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Synthesis of New Benzothiazole Derivatives and Evaluation of Cytotoxicity of N-(6-Substitued-1,3-benzothiazol-2-yl)-4-phenyl-1,3-thiazol-2(3H)-imine Compounds

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In this research, 2,3-cyclization of unsymmetrical thioureas affords N-(6-substitued-1,3-benzothiazol-2-yl)-4-phenyl-1,3-thiazol-2(3H)-imine compounds by the reaction of 1-(1,3-benzothiazol-2-yl)thiourea derivatives and 2-bromoacetophenone in the presence of Et_3N. Also, methyl-1,3-benzothiazol-2-yl carbamodithioate derivatives with methyl anthranilate were reacted and 3-(benzo[d]thiazol-2-yl)-2-thioxo-2,3-dihydroquinazolin-4(1H)- one derivatives as new compounds were synthesized. In the following, synthesized compounds (2a-2d) were evaluated for anti-tumor activity against MCF-7, MAD-MD-231, HT-29, HeLa, Neuro-2a, K-562 and L-929 cell lines, and the results obtained from MTT-assay revealed the best cytotoxicity for 2b compound containing a phenolic segment in their buildings. Apoptosis assay for this compound carried out by flow cytometry supported the other results.

Keywords: Unsymmetrical thioureas, MTT-assay, Flow cytometry, TUNEL assay, 1-(1,3-benzothiazol-2-yl)thiourea, 3-(benzo[d]thiazol-2-yl)-2-thioxo-2,3-dihydroquinazolin-4(1H)-one

INTRODUCTION

Thiazoles and their derivatives exhibit various biological activities such as antibacterial [1], anti-fungal [2], anti-inflammatory [3], anti-convulsant [4], pifithrin (pft-a), skin whitening agent (KHG22394) [5]. Furthermore, some thiazoles are used in agriculture as pesticides and plant growth regulators [6]. Several novel thiazole derivatives have been reported in the literature and evaluated as antimicrobial [7], antioxidant [8] and antibacterial agents [9].

Cancer is an enormous global health burden, touching every region and socioeconomic level. Todays, cancer accounts for one in every eight deaths worldwide - more than those due to combined HIV/AIDS, tuberculosis, and malaria. Cancer chemotherapy targeting tumor progression represents one of the most relevant challenges to the chemists and oncologists. Chemotherapy or medicinal agents used to destroy cancer cells are the current approaches in the treatment of malignancies. This therapeutic approach has aroused lots of interest amongst researchers and a great deal of efforts has now been focused on the design and development of a variety of anticancer drugs.

The above mentioned biological and synthetic significance of thiazole derivatives motivated us to carry out the synthesis of some new derivatives of benzothiazoles. Herein, we report synthesis of derivatives of N-(6-substitued-1,3-benzothiazol-2-yl)-4-phenyl-1,3-thiazol-2 (3H)-imine (2a-2d) (Scheme 1), and then survey the anticancer activities of these compounds.

In order to synthesize more new derivatives of benzothiazole, we reacted methyl-1,3-benzothiazol-2-yl carbamodithioate derivatives (3) with methyl anthranilate in ethanol under reflux condition as shown in Scheme 2.

The synthesis method as well as the spectral data of the 1-(6-substitued-1,3-benzothiazol-2-yl)thiourea (1) and methyl-1,3-benzothiazol-2-yl carbamodithioate derivatives

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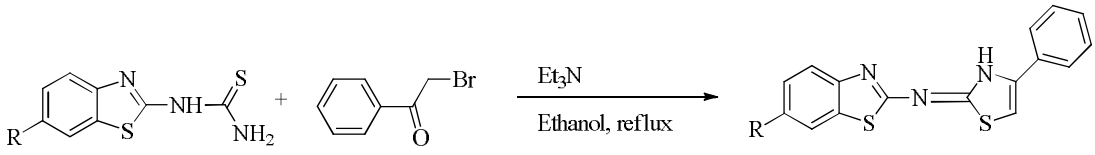


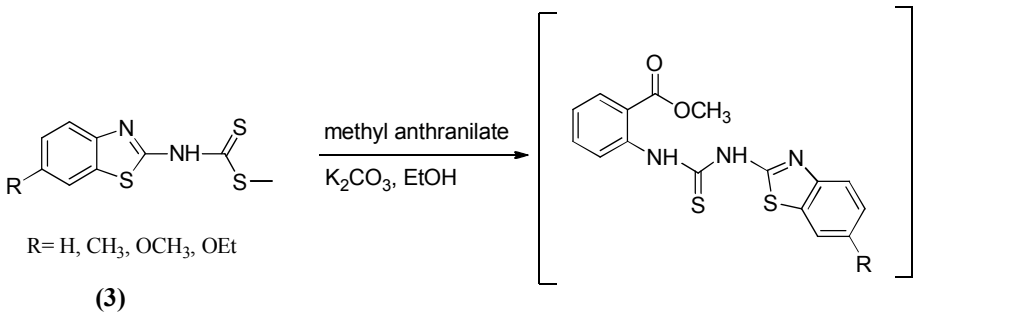
(1)

R= H, CH_3, OCH_3, OEt

(2a-2d)

R= H, CH_3, OCH_3, OEt

Scheme 1



R= H, CH_3, OCH_3, OEt

(3)

R= H, CH_3, OCH_3, OEt

(4a- 4d)

(5)

Scheme 2

(3) have been reported previously [10].

EXPERIMENTAL

Chemicals

All solvents, reagents and compounds were purchased from Merck and Fluka companies. RPMI-1640 medium, Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were purchased from GIBCO (Gaithersburg, USA). Penicillin and streptomycin were purchased from Biochrom AG (Berlin, Germany). MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a yellow tetrazole) was purchased from Sigma Co., Ltd. Cisplatin was purchased from Sigma Aldrich.

Apparatus

NMR spectra were recorded on Avance Brucker-400 MHz spectrometers. All chemical shifts in NMR experiments are reported as ppm and were referenced to residual solvent. FT-IR spectra were recorded on AVATAR-370-FTIR ThermoNicolet. All mass spectra were scanned on a Varian Mat CH-7 at 70 eV. Reaction was monitored by TLC using silica gel plates and the products were identified by comparison of their spectra and physical data with those of the authentic samples. Melting points were measured on an Electrothermal 9100 apparatus.

General Procedure for Synthesis of the N-(6-Substitued-1,3-benzothiazol-2-yl)-4-phenyl-1,3-thiazol-2(3H)-imine Derivatives (2a-2d)

To a solution of 1-(6-substitued-1,3-benzothiazol-2-yl)thiourea (1) (1 mmol), in absolute ethanol, 2-bromoacetophenone (1 mmol) and two drops of Et_3N as catalyst were added and the mixture was refluxed for several hours, a precipitate was formed. The mixture was filtered and washed with ethanol, then recrystallized from ethanol–water (1:1) to give the target compounds.

N-(1,3-Benzothiazol-2-yl)-4-phenyl-1,3-thiazol-2(3H)-imine (2a). ($C_{16}H_{11}N_3S_2$): Light yellow solid (90% yield), m.p.: 260 ºC; IR (KBr) cm^{-1}: 3161, 1600; ^1H NMR (400 MHz, DMSO) δ: 7.21-7.25 (t, *J* = 16.0 Hz, 1H aromatic); 7.33-7.37 (t, *J* = 16.0 Hz, 1H aromatic), 7.38-7.42 (t, *J* = 16.0 Hz, 1H aromatic), 7.45- 7.49 (t, *J* = 16.0 Hz, 2H aromatic), 7.56-7.58 (d, *J* = 8.0 Hz, 1H aromatic), 7.61 (s, CH, aliphatic), 7.90-7.92 (d, *J* = 8.0 Hz, 1H aromatic), 7.99-8.00 (d, *J* = 4.0 Hz, 2H aromatic), 12.44 (br s, 1H, NH); ^{13}C NMR (100 MHz, DMSO) δ: 107.39, 122.43, 123.15, 126.18, 126.83, 128.32, 129.22, 134.57, 149.95, 160.52; MS (m/z): 309 (M^+); Anal. Calcd. for $C_{16}H_{11}N_3S_2$: C, 62.11; H, 3.58; N, 13.58; S, 20.73. Found: C, 62.12; H, 3.21; N, 13.35; S, 21.32.

N-(6-Methyl-1,3-benzothiazol-2-yl)-4-phenyl-1,3-thiazol-2(3H)-imine (2b). ($C_{17}H_{13}N_3S_2$): Light Yellow solid (87% yield), m.p.: 245 ºC; IR (KBr) cm^{-1}: 3141, 1611; ^1H NMR (400 MHz, DMSO) δ: 2.40 (s, 3H, CH_3), 7.21-7.23 (d, *J* = 8.0 Hz, 1H aromatic), 7.34-7.38 (t, *J* = 16.0 Hz, 1H aromatic), 7.46-7.50 (t, *J* = 16.0 Hz, 3H aromatic), 7.62 (s, CH, aliphatic), 7.72 (s, 1H aromatic), 7.99-8.01 (d, *J* = 8.0 Hz, 2H aromatic), 12.55 (br s, 1H, NH); ^{13}C NMR (100 MHz, DMSO) δ: 21.33, 107.26, 122.20, 126.16, 127.99, 128.39, 129.27, 132.69, 134.55, 149.95, 160.52; MS (m/z): 323 (M^+); Anal. Calcd. for $C_{17}H_{13}N_3S_2$: C, 63.13; H, 4.05; N, 12.99; S, 19.83. Found: C, 63.36; H, 3.47; N, 13.03; S, 11.78.

N-(6-Methoxy-1,3-benzothiazol-2-yl)-4-phenyl-1,3-thiazol-2(3H)-imine (2c). ($C_{17}H_{13}N_3OS_2$): Yellow solid (85% yield), m.p.: 203 ºC; IR(KBr) cm^{-1}: 3113, 1601; MS (m/z): 339 (M^+).

N-(6-Ethoxy-1,3-benzothiazol-2-yl)-4-phenyl-1,3-thiazol-2(3H)-imine (2d). ($C_{18}H_{15}N_3OS_2$): Light Yellow solid (95% yield), m.p.: 219 ºC; IR (KBr) cm^{-1}: 3166, 1610; ^1H NMR (400 MHz, DMSO) δ: 1.34-1.38 (t, *J* = 16.0 Hz, 3H, CH_3), 4.05-4.10 (q, *J* = 20.0 Hz, 2H, CH_2), 6.98-7.01 (d, *J* = 12.0 Hz, 2H aromatic), 7.34-7.38 (t, *J* = 16.0 Hz, 1H aromatic), 7.46-7.50 (t, *J* = 16.0 Hz, 2H aromatic), 7.56 (s, 1H aromatic), 7.61 (s, CH, aliphatic), 7.99-8.01 (d, *J* = 8.0 Hz, 2H aromatic), 12.46 (br s, 1H, NH); ^{13}C NMR (100 MHz, DMSO) δ:; MS (m/z): 353 (M^+); Anal. Calcd. for $C_{18}H_{15}N_3OS_2$: C, 61.16; H, 4.28; N, 11.89; S, 18.14. Found: C, 61.29; H, 3.71; N, 11.74; S, 11.52.

General Procedure for Synthesis of the 3-(Benzo[d]thiazol-2-yl)-2-thioxo-2,3-dihydro-inazolin-4(1H)-one derivatives (4a-4d)

Methyl anthranilate (0.01 mol) and the methyl-1,3-benzothiazol-2-yl carbamodithioate derivatives (3) (0.01 mol) were dissolved in ethanol (20 ml). To this mixture, anhydrous potassium carbonate (100 mg) was added and refluxed for 18 h. The reaction mixture was cooled in ice and the solid separated was filtered and purified by dissolving in 10% alcoholic sodium hydroxide solution (95% alcohol was used for preparation) and re-precipitated by treating with dilute hydrochloric acid. The solid obtained was filtered, washed with water, dried and recrystallized from ethanol.

3-(Benzo[d]thiazol-2-yl)-2-thioxo-2,3-dihydro-quinazolin-4(1H)-one (4a). ($C_{15}H_9N_3OS_2$): Light yellow solid (yield 80%), m.p.: 265 ºC; IR (KBr) cm^{-1}: 3252, 1622, 1224; ^1H NMR (400 MHz, DMSO) δ: 7.37-7.42 (t, *J* = 16.0 Hz, 1H aromatic), 7.46-7.48 (d, *J* = 8.0 Hz, 1H aromatic), 7.53-7.61 (m, 2H aromatic), 7.82-7.86 (t, *J* = 16.0 Hz, 1H aromatic), 7.98-8.01 (d, *J* = 12.0 Hz, 1H aromatic), 8.05-8.08 (d, *J* = 12.0 Hz, 1H aromatic), 8.18-8.20 (d, *J* = 8.0 Hz, 1H aromatic), 13.33 (br s, 1H, NH); ^{13}C NMR (100 MHz, DMSO) δ: 116.23, 116.56, 123.26, 123.92, 125.33, 126.56, 126.78, 127.91, 136.79, 137.36, 140.17, 150.06, 159.00, 160.08, 175.23; MS (m/z): 311 (M^+); Anal. Calcd. for $C_{15}H_9N_3OS_2$: C, 57.86% H, 2.91%; N, 13.49%; S 20.60%. Found: C, 56.62%; H, 2.91%; N, 12.98%; S, 13.60%.

3-(6-Methylbenzo[d]thiazol-2-yl)-2-thioxo-2,3-di-hydroquinazolin-4(1H)-one (4b). ($C_{16}H_{11}N_3OS_2$): Yellow solid (yield 78%), m.p.: 260 ºC; IR (KBr) cm^{-1}: 3250, 1681, 1261; MS (m/z): 325 (M^+).

3-(6-Methoxybenzo[d]thiazol-2-yl)-2-thioxo-2,3-dihydroquinazolin-4(1H)-one (4c). ($C_{16}H_{11}N_3O_2S_2$): Yellow solid (yield 81%), m.p.: 258 ºC; IR (KBr) cm^{-1}:

3243, 1680, 1264; MS (m/z): 341 (M^+).

3-(6-Ethoxybenzo[d]thiazol-2-yl)-2-thioxo-2,3-dihydroquinazolin-4(1H)-one (4d). ($C_{17}H_{13}N_3O_2S_2$): Light yellow (yield 89%), m.p.: 240 ºC; IR (KBr) cm^{-1}: 3247, 1680, 1224; ^{1}H NMR (400 MHz, DMSO) δ: 1.47-1.51 (t, *J* = 16.0 Hz, 3H, CH_3), 4.11-4.16 (q, *J* = 20.0 Hz, 2H, CH_2), 7.14-7.16 (d, *J* = 8.0 Hz, 2H aromatic), 7.33-7.37 (m, 2H aromatic), 7.65-7.68 (t, *J* = 12.0 Hz, 1H aromatic), 7.99-8.01 (d, *J* = 8.0 Hz, 1H aromatic), 8.16- 8.18 (d, *J* = 8.0 Hz, 1H aromatic), 10.65 (br s, 1H, NH); ^{13}C NMR (100 MHz, DMSO) δ: 14.77, 64.20, 104.97, 115,10, 115.94, 116.56, 125.03, 125.38, 128.91, 136.33, 138.27, 138.80, 144.40, 154.56, 157.90, 175.69; MS (m/z): 355 (M^+).

General Procedure for Synthesis of the 3-(Benzo[d]thiazol-2-yl)-2-(methylthio)quinazolin-4(3H)-one (5)

3-(Benzo[d]thiazol-2-yl)-2-thioxo-2,3-dihydro-quinazolin-4(1H)-one (4a) (0.01 mol) was dissolved in 40 ml of 2% alcoholic sodium hydroxide solution (95% alcohol was used for preparation). To this dimethyl sulfate (0.01 mol) was added dropwise with stirring. The stirring was continued for 1 h, the reaction mixture was then poured into ice water. The solid obtained was filtered, washed with water, dried under high vacuum and recrystallized from ethanol/chloroform (75:25) mixture.

3-(Benzo[d]thiazol-2-yl)-2-(methylthio)quinazolin-4(3H)-one (5). ($C_{16}H_{11}N_3OS_2$): White solid (yield 75%), m.p.: 240 ºC; IR (KBr) cm^{-1}: 1702; ^{1}H NMR (400 MHz, DMSO) δ: 2.61 (s, 1H, SCH_3), 7.45-7.47 (t, *J* = 8.0 Hz, 1H aromatic), 7.52-7.56 (t, *J* = 16.0 Hz, 1H aromatic), 7.58-7.62 (t, *J* = 16.0 Hz, 1H aromatic), 7.66-7.68 (d, *J* = 8.0 Hz, 1H aromatic), 7.77-7.81 (t, *J* = 16.0 Hz, 1H aromatic), 7.97-7.99 (d, *J* = 8.0 Hz, 1H aromatic), 8.16- 8.18 (d, *J* = 8.0 Hz, 1H aromatic), 8.26-8.28 (d, *J* = 8.0 Hz, 1H aromatic), (^{13}C NMR (100 MHz, DMSO) δ: 15.35, 119.17, 121.98, 124.50, 126.41, 126.59, 126.71, 127.37, 135.40, 136.95, 147.37, 149.92, 155.85, 156.06, 161.55; MS (m/z): 325 (M^+).

BIOLOGICAL STUDIES

Cell Culture Methods

Human breast cancer cells MDA-MD-231 (ATCC HTB-26), human breast cancer cells MCF-7 (ATCC HTB-22), human cervix epithelial carcinoma HeLa (ATCC CCL-2), human leukemia cell line K-562 (ATCC CCL-243), human colon cancer cell line HT-29 (ATCC HTB-38), mouse neuroblastoma cell line Neuro-2a (ATCC CCL-131), and mouse fibroblast L-929 cell line (ATCC CCL-1) were obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA) and cultured at 37 °C in a humidified atmosphere of 5% CO_2 in air. HeLa cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) with 0.1 mM nonessential amino acids, 2 mM L-glutamine, 1.0 mM sodium pyruvate and 5% fetal bovine serum, at 37 °C in an atmosphere of 5% CO_2. Cells were plated in 96-well sterile plates at a density of 1×10^4 cells/well in 100 µl of medium and incubated for 24 h. Also MDA-MD-231, HT-29, MCF-7 and Neuro-2a were cultured in DMEM containing 10% fetal bovine serum, 100 units/ml of penicillin and 100 µg ml^{-1} of streptomycin. K-562 and L-929 cells were cultured in RPMI-1640 medium containing 10% fetal bovine serum, 100 units/ml of penicillin and 100 µg ml^{-1} of streptomycin.

MTT Assay in Human Cancer Cell Lines

Synthesis compounds were screened for antitumor activity against human breast cancer cells (MDA-MD-231) human cervix epithelial carcinoma (HeLa), human colon cancer cell line (HT-29), human breast cancer cells (MCF-7), mouse neuroblastoma cell line (Neuro-2a), mouse fibroblast L-929 cell line and using cisplatin as a comparative standard. Cell viability was evaluated using a colorimetric method based on the tetrazolium salt MTT([3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]), reduced by living cells to yield purple formazan crystals. Cells were seeded in 96-well plates at a density of 2-5 $\times 10^4$ cells of MDA-MD-231, MCF-7, HeLa, HT-29, Neuro-2a and L-929 per well in 200 µl of culture medium and left to incubate overnight for optimal adherence. After careful removal of the medium, 200 µl of a dilution series of the compounds in fresh medium were added and incubation was performed at 37 °C/5% CO_2 for 72 h. Compounds (2a-2d) were first solubilized in DMSO, diluted in medium and added to the cells in final concentrations between 20 nM and 200 µM. The percentage of DMSO in cell culture medium did not exceed 0.3%. Cisplatin was first solubilized

in saline and then added at the same concentrations used for the other compounds. At the end of the incubation period, the compounds were removed and the cells were incubated with 200 µl of MTT solution (500 µg ml^{-1}). After 3-4 h at 37 °C/5% CO_2, the medium was removed and the purple formazan crystals were dissolved in 200 µl of DMSO by shaking. The cell viability was evaluated by measurement of the absorbance at 570 nm by using a STAT FAX-2100 microplate reader (Awareness Technology, Palm City, FL, USA). The cell viability was calculated dividing the absorbance of each well by that of the control wells (cells treated with medium containing 1% DMSO). Each experiment was repeated at least three times and each point was determined in at least three replicates.

Apoptosis Assay for Compounds 2b by Flow Cytometry

In order to study the way in which compounds 2a-2d are produced, the cellular death (necrosis or apoptosis) studies of flow cytometry were performed on the synthesized compounds, and cisplatin as a reference. These compounds were incubated for 24 h at a concentration close to IC_{50}, and the results are shown in Fig. 1. Four areas in the diagrams stand for necrotic cells (Q1, low Annexin V-FITC and high PI signal, left square on the top), late apoptosis or necrosis cells (Q2, high Annexin V-FITC and high PI signal, right square on the top), live cells (Q3, low Annexin V-FITC and low PI signal, left square at the bottom), and apoptosis cells (Q4, high Annexin V-FITC and low PI signal, right square at the bottom), respectively. As shown in Figure 1 and Table 2, compound 2b could induce apoptosis against MDA-MB-231 cancer cell lines. However, the proapoptotic property needs further investigation to better understand the precise mechanism of action of the complexes.

Analysis of Apoptosis for Compounds 2a-2d by Cytometry Using TUNEL Assay

Apoptosis was also detected using an *in situ* cell death detection kit (Boehringer Mannheim Corp., Indianapolis, IN) as described by Narla et al. and Zhu *et al*. [11,12]. Cells were incubated with compounds 1-3 in 0.3% DMSO or 1:16-diluted plasma samples from DFX-treated mice for 48 h at 37 °C, and were fixed, permeabilized, incubated with the reaction mixture containing TdT- and FITC-conjugated dUTP, and counterstained with propidium iodide. Cells were transferred to slides and viewed with a confocal laser scanning microscope (Bio-Rad MRC 1024) mounted on a Nikon Eclipse E800 series upright microscope as reported previously [11,12].

Statistical Analysis

The IC_{50} values were expressed as mean ± standard deviation (SD) from at least three independent experiments. Statistical tests including one-way analysis of variance, ANOVA, Tukey multiple comparison or unpaired Student's *t* tests were performed using SPSS, ver.17 software. A *p* value of less than 0.05 was considered significant.

ANTI-TUMOR ACTIVITY

The *in vitro* cytotoxiciy of these compounds, against MDA- MDA-MD-231, MCF-7, HeLa, HT-29, Neuro-2a and L-929 cell lines were determined by MTT-based assays (Table 1). Cytotoxic activity was simultaneously measured for mouse fibroblast normal cell line (L-929) as control. As shown in Table 1, compounds 2a-2d displayed cytotoxic activity against L-929 significantly higher than cancer cell lines making them as the appropriate candidates for anti-cancer drug. A notable point is that in the case of cisplatin, the IC_{50} value against normal cell L-929 was so low that it was unable to make a distinction between normal and cancer cell, while mentioned shortcoming was overcome using compounds 2a-2d.

The IC_{50} values in all cell lines were measured for the synthesized compounds as well as cisplatin as a comparative standard. The measurements were carried out after 72 h of incubation using concentrations of the compounds in the range 20 nM and 200 µM. The determined values of IC_{50} for compounds 2a-2d were spanned between 5.94 and up to 100 µM, while those found for cisplatin as a comparative standard ranged between 0.7 an up to 100 µM (Table 1).

In addition, based on the obtained results, 2b exhibited maximum cytotoxicity. An interesting point is that both of them are involved in hydroxyl-group disposing to show excellent anti-tumor activity. Compound 2b, displaying the best cytotoxicity against cancer cell lines, were selected for apoptosis assay by flow cytometry. The results are given in

Table 1. Anticancer Activity of Derivatives (2a-2d) against HeLa, K-562, MCF-7, MDA-MB-231, HT-29, Neuro-2a and L-929 Cell Lines after 72 h of Continuous Treatment

	$IC_{50} \pm SD$ (μM)[a]						
Compound	MCF-7	MDA-MD-231	HT-29	HeLa	Neuro-2a	K-562	L-929
2a	53.1 ± 6.93	31.6 ± 3.11	67.3 ± 5.23	37.2 ± 2.80	76.5 ± 7.92	78.3 ± 7.05	> 100
2b	17.5 ± 3.41	5.94 ± 1.98	21.5 ± 2.55	46.2 ± 5.71	83.2 ± 9.29	25.6 ± 2.45	> 100
2c	49.9 ± 5.0	48.1± 4.47	29.4 ± 3.19	35.3 ± 4.45	74.6 ± 5.53	47.1 ± 3.94	> 100
2d	66.5 ± 6.23	46.2 ± 4.75	62.4 ± 5.55	63.1 ± 5.34	88.4 ± 5.63	65.2 ± 5.81	> 100
Cisplatin	6.17 ± 2.26	21.5 ± 2.76	17.2 ± 2.56	0.45 ± 0.15	70.9 ± 8.56	24.8 ± 4.2	0.9 ± 0.1

[a]The concentration of the complex required to inhibit cell growth by 50%. The experiments were done in triplicate. Data were expressed as the mean of the triplicate. The agent with IC_{50} > 100 μM is considered to be inactive.

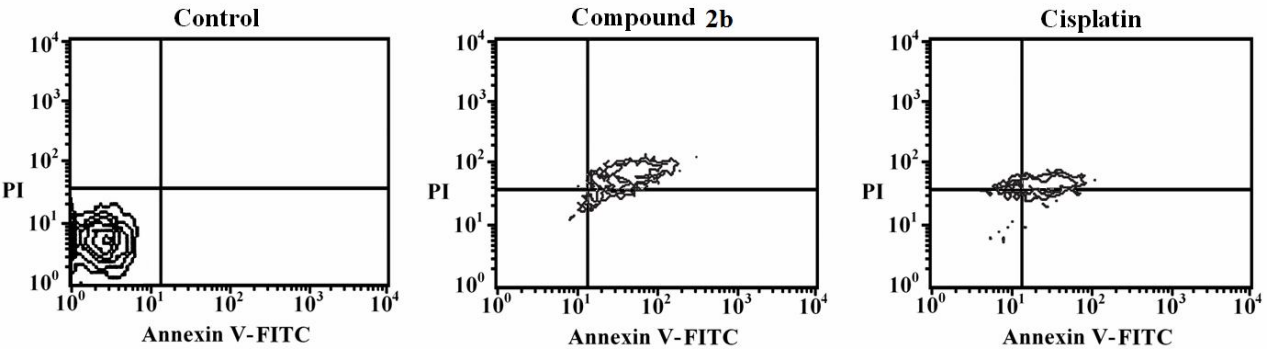


Fig. 1. Flow cytometric results after the exposure of MDA-MD-231 cancer cells to the active compound 2b and cisplatin. Four areas in the diagrams represent four different cell states: necrotic cells (Q1), late apoptotic or necrotic cells (Q2), living cells (Q3), and apoptotic cells (Q4).

Fig. 1 and Table 2.

Clearly seen from Fig. 1 and Table 2, compound 2b showed a high population of apoptotic cell (94.8%) and nearly 1.2-fold higher than cisplatin (78.9%) at the same concentration. The synthesized compound (2b) also induced cell death was confirmed to be apoptotic using the TUNEL of exposed 3'-OH termini of DNA with dUTP-FITC. As shown in the confocal laser scanning microscopy images in Fig. 2, compound-treated MDA-MB-231 (or K-562) cancer cells, examined for dUTP-FITC incorporation (*green fluorescence*) and propidium iodide counterstaining (*red fluorescence*) exhibited many apoptotic yellow nuclei (*superimposed green and red fluorescence*) at 24 h after treatment.

The results demonstrated that the newly synthesized compounds could induce apoptosis against MDA-MD-231 cancer cell lines. However, the pro-apoptotic property needs further investigation to better understand the precise mechanism of action of these compounds, and basic pre-clinical research is needed before they could be recommended for human administration.

CONCLUSIONS

In summary, design, synthesis and *in vitro* anti-tumor activity of N-(6-substitued-1,3-benzothiazol-2-yl)-4-phenyl-

Table 2. Percentages of the Cell Death Pathways Observed by the Flow Cytometry Assay for Compound 2b

Treatment	%Vital cells	%Apoptotic cells	%Late apoptotic/necrotic cells	%Necrotic cells
Control	100	0	0	0
Cisplatin	9.4	35.7	43.2	11.7
Compound 2b	4.4	31.2	63.6	0.8

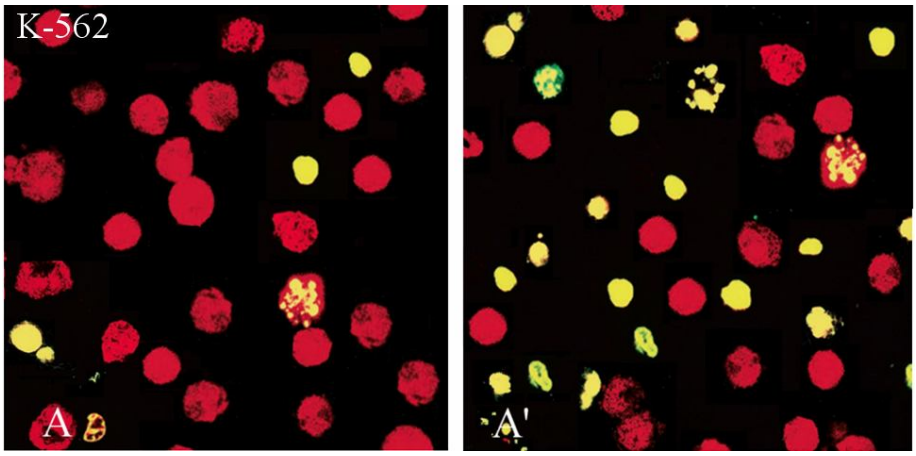


Fig 2. Compound 2b induces apoptosis in human breast (MDA-MD-231) cancer cell lines. MDA-MD-231 breast cancer (A, A' for 2b) cells were incubated with 10 mM of 2b for 24 h, fixed, permeabilized, and visualized for DNA degradation in a TUNEL assay using dUTP-labeling. Red fluorescence, nuclei stained with propidium iodide. Green or yellow (*i.e.*, superimposed red and green) fluorescence, apoptotic nuclei containing fragmented DNA. When compared with controls, treated with 0.3% DMSO (A), several of the cells incubated with 2b (A') exhibited apoptotic nuclei.

1,3-thiazol-2(3H)-imine derivatives were described. These derivatives were prepared in favorite yields. The *in vitro* anti-tumor effects on seven cancer cell lines were measured and displayed moderate to high activities. Moreover, compared to cisplatin as a well-known anti-cancer drug, some derivatives exhibited very high activity against cancer cell lines. Additionally, in most cases, the cytotoxicity for normal cell line was significantly higher than that for the cancer cells, making them susceptible for *in vivo* evaluations. Compound 2b, showing the best anti-tumor effects.

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